IV-D 1

D. Site of Origin of Lung Cancers

This section relates to studies that have endeavored to trace the actual site of origin of respiratory cancers as well as papers that describe clinical observation of the development of lung cancer at its onset.

NEW LIGHT ON THE ORIGINS OF LUNG CANCER

Leo G. RIGLER, M.D., of Cedars of Lebanon Hospital and the University of California in Los Angeles, was frequently successful in a prolonged search for earlier chest x-rays of patients found to have cancer of the lung (11-r):

> "It is significant that in the approximately 100 such patients carefully studied, all but two showed evidences of an abnormality in the area of the lung later proved to be the location of the carcinoma.... In this series, it is clear that more than 50% of the cases exhibit roentgen evidences of the disease process more than two years prior to either the appearance of symptoms or the determination of a definitive diagnosis....
>
> "It is important that we revise our ideas about both the origin and the rapidity of growth of carcinoma of the lung. Our own evidence indicates clearly that the majority of all cases arise in a branch bronchus rather than in a major bronchus and that in the majority of cases there is a relatively slow course of events."

IV-D 3

HIGH FREQUENCY OF METASTASES OF CANCER TO LUNGS

Averill A. LIEBOW, M.D., Professor of Pathology, Yale University School of Medicine, and Consultant Pathologist to the Armed Forces Institute of Pathology, is an authority on the pathologic aspects of lung cancer. He has labored for many years to bring order into the highly disorganized nomenclature that has arisen all over the world. In 1956 (14-1) he cautioned on the need to distinguish between metastases and primary lung cancer.

> "Since the pulmonary capillaries act as a filter interposed between the systemic veins and the left heart, and since the lymphatic connections of the lung with the mediastinum also are rich, it is not surprising that the lungs should be the seat of metastases even more frequently than of primary tumors....
>
> "Whenever multiple tumors are demonstrated within the lung, they should first and foremost be considered metastatic until proved otherwise....
>
> "Metastases may sometimes present themselves as large isolated masses, occasionally many years after the extirpation of a distant primary tumor...
>
> "Confusion with primary neoplasms may occur, since some metastatic tumors may involve bronchi, even of major size."

BRONCHIAL CILIA DESTROYED BY LUNG INFECTIONS

George N. PAPANICOLAOU, M.D., Ph.D., of Cornell University Medical College, New York City, in 1960 (46-p) reported that extensive degeneration of the ciliated cells of the bronchial mucosa was a persistent feature in the sputum smears of the patient during the ten months preceding the appearance of cells suggestive of cancer.

> "This degenerative process is referred to as CCP, an abgreviation of the term 'ciliocytophthoria,' which means ciliated cell destruction. An intensive study of this process, which is usually observed in the sputum smears of patients with acute and chronic pulmonary infections, has been underway for the past three years as a co-operative project involving our Research Laboratory, the Strang Clinic of Memorial Center and the Rockefeller Institute with the support of a grant from the American Cancer Society.
>
> "The results of these investigations based on a statistical analysis of 1000 cases, both asymptomatic and symptomatic, indicate that this phenomenon of mass degeneration of the ciliated cells is definitely related to the presence of chronic and acute inflammatory disease.
>
> "In our series, the greatest loss of ciliated cells was found to occur in some of the acute cases observed during the 1957 virus epidemic of Asiatic influenza. More substantial evidence in favor of the view that these degenerative changes affecting the ciliated cells are linked to a viral etiologic factor has been provided by the observations of Pierce and Hirsch of the Rockefeller Institute (48-p) who also stated that in their own series this CCP phenomenon was noted in a high proportion of individuals with proven viral respiratory infection in contrast to its relatively rare occurrence in patients with acute or chronic pulmonary disease of non-viral etiology....

"In a tabulation of 500 largely symptomatic cases made by my two research associates in the study... the numerical CCP index reached a significantly higher level not only in the inflammatory group as compared with the normal but also in the group of the 70 primary carcinomas of the lung included in this series....

"These observations are strongly suggestive of a certain relationship between the chronic or acute inflammatory disease and a subsequent development of cancer of the lung. A similar conclusion has been reached by a number of clinical investigators who provided a well documented evidence in favor of this view....

"In view of the fact that in a single day of an acute pulmonary inflammatory illness the destruction of ciliated cells as proven by the microscopic examination of large numbers of sputum specimens, may be conservatively calculated in certain cases to be approached or even surpassing the one million mark, it is reasonable to assume that such an extraordinary depletion of the ciliated components of the bronchial epithelium would, in all probability, result in the formation of non-ciliated areas, which most likely would tend to cause stasis and accumulation of tar or any other inhaled carcinogenic substances.

"The loss of such a stupendous number of cells during the whole period of an acute illness and of the often persisting chronic condition could hardly be fully compensated by the active proliferation of the basal cells of the bronchial mucosa, particularly in persons of advanced age, whose cells and tissues have a decreased regenerative potential....

"Acute and prolonged chronic pulmonary infections are very likely to cause a severe depletion of the ciliated components of the bronchial epithelium and the formation of deciliated islands thus opening the gateway to the action of various extraneous carcinogenic factors.... The acceptance of such an interpretation would certainly tend to indicate that one of our best control weapons against lung cancer is perhaps a systematic and well organized campaign against these chronic and prolonged forms of pulmonary infectious disease."

BABY LUNG CANCERS FOUND IN SMALLER BRONCHI

Doctors Arthur B. PETERSEN, Warren C. HUNTER and Vinton D. SNEEDEN of the Department of Pathology, University of Oregon Medical School, at Portland, reported in 1949 (16-p) these observations on minute tumors in small bronchi:

> "Five examples of minute and accidentally discovered pulmonary growths found in routine autopsy sections have been studied with the hope of discovering the histogenesis of primary neoplasia. All involved quite small bronchi....
>
> "The presence of localized bronchiectasis and fibrosis in four of the five small tumors affords a possible clue as to altered physiology and structure which might serve as a predisposing cause for neoplasia...."

PERIPHERAL ORIGINS OF LUNG CANCER DEMONSTRATED

Dr. C. RAEBURN, of the Area Pathological Laboratory, Whipps Cross Hospital, and Dr. Herbert SPENCER, of the Department of Pathology, St. Thomas's Hospital Medical School, both of London, England, reported in 1953 (1-r) on a study on the origin and development of lung cancer. They said:

> "Hitherto it has been customary to regard most lung carcinomata as having arisen from main stem bronchi and their immediate divisions, largely because the main bronchi so frequently become involved in a mass of growth. Although main bronchi undoubtedly can be the seat of origin of primary lung carcinomata, where we have been able to procure very small early growths before spread has occurred, the growths have in every case been of the intra-epithelial variety analogous to Bowen's disease of the skin, intra-epithelial cancer of the cervix uteri and esophagus, and Paget's intra-epithelial cancer of the breast.
>
> "Such carcinomata undoubtedly can metastasize and then usually behave as a fairly rapidly spreading variety of squamous-cell carcinoma. Apart from this type of growth, which starts ab initio as multiple areas of epithelial hyperplasia, all the other small growths which have been obtained from lungs show unmistakable evidence of having arisen in and about damaged lung tissue....
>
> "The relationship of previous lung damage to primary malignant disease of the lung has been noted before, but the present tendency is to regard most lung tumors as having arisen from the lobar bronchial epithelium or glands. From our experience it would appear possible that some growths start in the lung substance from bronchiolar tissue in areas of chronic fibrosis and hence spread to the regional hilar glands. From the regional hilar glands subsequent backspread into the lymphatic channels in the adjacent lobar bronchial wall may then occur.

"The almost universal finding of at least one or two scarred areas in the lung substance at post-mortem examination contrasts with the rarity of naked-eye and even microscopical evidence of chronic damage to the main bronchi and their surface epithelium.

"Careful comparison of the structure of scar cancer and simple lung scars, and the ability to demonstrate all stages leading to malignant change in the latter, has convinced us, apart from other reasons, that the scarring antedated the appearance of malignant changes."

FIBROTIC CHANGES IN LUNG TISSUE

"There is no reason to believe that malignant cells in alveolar spaces of the lungs cannot arise in more than one way," A. E. ANDERSON Jr., M.D., and Alvan G. FORAKER, M.D., of the Baptist Memorial Hospital of Jacksonville, Fla., said in a 1960 paper (15-a). Although they cited authors who disagree on this point, without taking sides, they presented a description of pulmonary fibrosis arising from a multitude of causes and occasioning a multitude of respiratory disorders, including cancer.

> "Pulmonary fibrosis of some degree is extremely common. It may be an incidental postmortem finding or it can be reflected as major clinical disease.... Despite its frequency, there has been relatively little interest in the detailed pathology of pulmonary fibrosis. Thus, the exact role of the elastic fibers in the pathogenesis of emphysema is still unsettled, although these structures can be easily demonstrated with appropriate stains. Moreover, established concepts often have been disregarded in the interpretation of clinical material....
>
> "Liebow, Loring and Felton (32-1) have written a careful review on the musculature of the lungs in chronic pulmonary disorders. Thus, abnormal proliferation has been described in a wide variety of unrelated conditions, including emphysema, bronchiectasis, lung abscess, asthma, bronchitis, bronchiolitis, tuberculosis, tumor, 'brown induration' of lungs, 'honeycomb' lungs, congenital cysts, 'bronchiolar emphysema,' and chronic passive congestion...."

DISTRIBUTION OF INHALANTS IN LUNGS NOT PRINCIPAL LUNG CANCER FACTOR

Doctors Arend BOUHUYS, of University Hospital, Leiden, the Netherlands, and Gunnar LUNDIN, of the University of Lund, Sweden, in 1959 (53-b) reviewed 99 papers by authors in many countries on distribution of inspired gas in the lungs, including their own experiments at the Institute of Physiology in Lund:

> "It seems likely that the distribution of ventilation and blood flow within the lungs are even more dynamic processes than is apparent from the experimental results.... The higher ventilation/capillary blood flow ratios in the upper lobes result in higher alveolar oxygen tensions, which may bear some relation to the predominantly apical localization of phthisis. The higher ventilation rate of the lower lobes, on the other hand, would suggest that these lobes would be more exposed to inhaled pathogenic agents such as silica dust and carcinogens (Martin & Young, 1956) (47-m).
>
> "In 2145 cases of bronchial carcinoma, however, 55.4% occurred in the upper lobes, 29.4% in the lower lobes and the remaining in the middle lobe and in the main bronchi (Victor, 1955) (7-v), which suggests that the distribution of inhaled carcinogens in the lungs is not the most important factor in the pathogenesis of bronchial carcinoma."

For other discussions of cell changes in the respiratory tract see 32-c, 18-c, 12-o, 6-o, 46-p, 35-p, 48-p, 28-r, 20-r, 27-s, 6-w, 42-w, 33-w, 34-w, 35-w, 36-w, 38-w

For further references relating to classification of tumor cells see 3-b, 4-b, 73-b, 38-b, 4-c, 6-f, 7-f, 8-f, 66-m, 8-p, 1-r, 2-r, 3-r, 4-r, 5-r, 26-r, 20-r, 9-t, 41-w, 22-w

V. WHAT ARE THE RESULTS OF EXPERIMENTS?

The principal experiments to test the hypothesis that tobacco use might be linked with human lung cancer have been with laboratory animals.

Exposure of animals to inhalation of tobacco smoke has uniformly failed to induce lung cancers even in the most susceptible species. However, tests with other inhaled substances have shown that lung cancer can be induced in both susceptible and resistant strains by inhalation (19-a, 9-k, 19-k).

The application of large doses of machine-produced tobacco smoke condensates to the shaved backs of mice has resulted in the reporting of cancers of the skin in some exposed animals in a few laboratories. Not all strains of mice react to lifetime exposures to such condensates, nor are all species of animals affected by the tobacco painting technique.

Scientists discuss the various substances, including natural sugars, that can induce skin cancer in animals by painting, injection, or otherwise. They also caution against uncritical extrapolation of animal results to man.

Modern methods of chemical analysis of the smoke of cigarettes and other tobacco have resulted in reports of the detection of the presence of extremely minute quantities of substances which, in pure and concentrated forms, have been shown to produce cancer on test animals. None of these substances have been detected in sufficient quantity, either alone or in combination with others, to account for even the limited cancer-producing results obtained on the skins of mice. They have not been shown to cause cancers of any type in man, nor in primates.

The failures of multiple attempts to produce cancer with various tobacco compounds applied to fetal lung tissues implanted in living hosts are significant, even though the same tissues were repeatedly and highly sensitive to known carcinogens in the same laboratory.

There has even been work demonstrating that tobacco tar has inhibited the growth of an experimental cancer under certain conditions.

For convenience, comments on these matters have been grouped as follows:

A. Smoke Inhalation Tests

B. Injection and Implant Tests

C. Species Differences

D. Applicability to Humans

E. Chemistry of Smoke

REVERSIBLE CHANGES IN RESPIRATORY TRACT OF MICE EXPOSED TO CIGARETTE SMOKE

A. Smoke Inhalation Tests

A study has been carried on for several years by Cecilie LEUCHTENBERGER, Ph.D., Rudolf LEUCHTENBERGER, M.D., William ZEBRUN, Ph.D., and Patricia SHAFFER at the Institute of Pathology, Western Reserve University, at Cleveland, Ohio, the two principal authors continuing this work at the Children's Cancer Research Foundation, Boston, Mass. In 1960 (33-1) they summarized their findings as follows:

> "Further correlated histological, cytological and cytochemical studies of bronchi from mice exposed to cigarette smoke resulted in the following observations:
>
> "1. Regardless of dose or time of exposure to cigarette smoke, there was extreme variability of response from mouse to mouse ranging from 'no alterations' to bronchitis associated with atypical epithelial proliferation.
>
> "2. No relationship between severity or frequency of bronchial lesions and dose or time of exposure to cigarette smoke was found.
>
> "3. There was an absence of invasive bronchogenic carcinoma in mice exposed to cigarette smoke, even in those that had nearly life span exposure.
>
> "4. There was a decrease in the frequency of bronchial lesions, suggesting reversibility, after cessation of exposure.
>
> "5. An early increase of intranuclear protein (before microscopic alterations) was followed by a gradual increase of deoxyribonucleic acid (DNA) content.
>
> "6. There was a return to normal intranuclear protein and DNA content, suggesting reversibility of cytochemical changes after cessation of exposure."

In their discussion, the authors said:

> "While it is impossible, a present, to relate any particular factor or factors of the host to the resistance or susceptibility of the tracheobronchial tree to cigarette smoke, the investigation of agents possibly carried by the host, which may contribute to the injury of the bronchi, would seem of great interest. Among the many possible host factors, viruses deserve special consideration, the more so since they occur with a certain frequency in mice and are capable of producing respiratory lesions.
>
> "It is well known that mice may be carriers of latent viruses that, under certain circumstances, may provoke alterations.... There are two main lines that we are investigating at present. The first is concerned with the detection of latent viruses in mice prior to exposure to cigarette smoke... The second line is concerned with the effect of exposure to virus in addition to exposure to cigarette smoke.
>
> "If our concept is correct -- that viruses may act as cofactors in the production of the bronchial lesions -- then mice that carry latent viruses prior to the exposure to smoke, or that are infected with vuruses, should develop frequent and perhaps more severe lesions, while mice free of virus should either be refractory or should develop only mild lesions."

NEGATIVE BRITISH RESULTS

Professor R. D. PASSEY, M.D., of the Chester Beatty Research Institute of Cancer Research at the Royal Cancer Hospital, of London, England, said in 1958 (6-p):

> "The lack of success of many experiments with cigarette smoke and its products to throw light on the problem of the association of lung cancer with cigarette smoking has led to the suggestion that the association is an indirect one, and that some indirect approaches might lead to useful knowledge in this field. Several such approaches are being investigated at the moment. Our failure during the past five years (as recorded in previous Annual Reports) to induce lung tumors in mice, rats and hamsters by exposure to strong concentrations of cigarette smoke is a striking negative result. In these experiments the only lung cancer encountered has been a solitary tumor in a control rat. This tumor was associated with marked bronchiectasis. This has led to the careful examination of old breeding males, not subject to any smoking hazard, and six additional tumors have now been encountered in untreated animals. It is noteworthy that in each of these rats marked bronchiectasis has been an associated condition. The difficulty in the interpretation of this association is that, as in research institutes elsewhere, it is rare to make a post-mortem on any old rat without finding some more or less severe chronic bronchial lesions."

OTHER TESTS & RELATED COMMENTS

Dr. Harold L. STEWART, Chief of the Pathologic Anatomy Branch, National Cancer Institute, of Bethesda, Md., was asked about animal smoke inhalation tests in Congressional hearings in 1960 (54-s). He replied:

> "I did an experiment on that a number of years ago in which mice were exposed to cigarette smoke in a chamber for many months and as a matter of fact, the experimental animals had less lung cancer than the control animals, so you can understand my natural reluctance to accept the statement of many of my colleagues that cigarette smoking is the cause of lung cancer in human beings."

Dr. B. M. WRIGHT, of the Pneumoconiosis Research Unit, Llandough Hospital, Penarth, Wales, in a 1955 letter (26-w) said that he had concluded experimental work in smoke exposure with negative results, but had not found time to publish them. He said:

> "Briefly, the experiments consisted of the exposure of two groups of Strong 'A' strain mice to the inhalation of the diluted smoke of 60 cigarettes over 24 hours, smoked at the rate of three an hour.... Apart from a striking effect on the growth rate of the exposed mice, no significance in the incidence of lung tumors was observed over a period of exposure up to 15 months."

Richard DOLL, O.B.E., D.Sc., M.D., FRCP, Member of the Statistical Research Unit, Medical Research Council, said in "Carcinoma of the Lung," published in 1958 (16-d):

> "Experiments in which animals were exposed to the tar or smoke of tobacco have uniformly failed to produce any pulmonary tumors comparable to the bronchial carcinoma of man."

W. CARRUTHERS, B.Sc., Ph.D., of the British Medical Research Council, Carcinogenic Research Group, University of Exeter, England, wrote in 1958 (3-c):

> "The fact that a given material will produce skin cancer in mice or rabbits is far from being presumptive evidence that the same material can cause lung cancer in man. Apart from some experiments with radioactive substances by Lisco and Finkel,* bronchial carcinoma of the type which occurs in man has never been produced in laboratory animals."* (37-1)

J. W. COOK, D.Sc., Ph.D., F.R.S., FRIC, Vice-Chancellor of the University of Exeter, England, writing in 1957 (13-c) said:

> "The lung tumors which have been induced in animals with chemical carcinogens are usually adenomas, and tumors analogous to the bronchocarcinoma of man have not so far been induced experimentally in animals."

G. Burroughs MIDER, M.D., of the National Cancer Institute, Bethesda, Md., said in 1956 (29-m):

> "Few pulmonary cancers at all comparable to human types of bronchogenic carcinoma have been produced in laboratory animals.... The lung is an important excretory organ and the possible production of pulmonary cancers by agents entering the body through extrarespiratory channels cannot be disregarded."

Jacob FURTH, M.D. and John L. TULLIS, M.D., of the Children's Cancer Research Foundation and Cancer Research Institute of the New England Deaconess Hospital, of Boston, Mass., discussed carcinogenesis by radioactive substances in 1956 (18-f). They said:

> "The carcinogenic response varies so widely with species and strains that the liability of man to develop certain neoplasms will be learned only from observations on man. Animal experiments help in the understanding of the underlying basic mechanisms and in obtaining leads on possible events, but direct extrapolation of data among species is not permissible."

Marvin C. KUSCHNER, M.D., of New York University-Bellevue Medical Center, reported in 1956 (13-k) on various experimental methods in which attempts were made to induce lung cancer in mice with methylcholanthrene, a strong carcinogen when applied to the skin of these animals. Tobacco was not involved. He said:

> "A substantial uncertainty stands in the way of the experimental evaluation of suspected carcinogens for the respiratory system at the present time as a result of the fact that the kind of cancer generally seen in man has rarely been observed in experimental animals.... Bronchogenic carcinoma comparable to that seen in humans has been produced by two... procedures, thread transfixion and pellet implantation, but not as yet by inhalation or intratracheal injection."

M. B. SHIMKIN, E. C. HAMMOND, and E. L. WYNDER collaborated in a study of animal experiments and other data in 1959 (15-c). Quotations relevant to the present subject follow:

> "It is, of course, a fact that many agents shown to be carcinogenic to the skin of mice have not been proved carcinogenic to man.... The pulmonary adenomatous tumor in mice, rats, and guinea pigs cannot be compared with the bronchogenic carcinoma in man."

Frank Philip COLEMAN, M.D., Assistant Professor of Clinical Surgery, Medical College of Virginia, at Richmond, wrote in 1954 (37-c):

> "The etiology of cancer of the lung remains as obscure as that of cancer elsewhere in the body. It has been suggested that excessive smoking carried out over a long period of time, twenty years or more, may be a causative factor in its production. So far, our laboratory has been unable to produce cancer of the respiratory tract in animals by the inhalation of tobacco smoke over a period of years. (Harvey B. Haag, personal communication). The questionable carcinogenic agent released by smoking cigarettes has not been identified and must await experimental proof before it can be accepted as a cause of lung cancer."

V-B

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B. Injection and Implant Tests

NO TOBACCO CANCERS WERE PRODUCED IN LUNG TISSUE TRANSPLANTS

Harry S. N. GREENE, M.D., Professor and Chairman of the Department of Pathology at Yale University School of Medicine, in New Haven, Conn., in 1957 (27-g) described tests of tobacco on living lung tissue as follows:

> "Cancer is not limited to man but occurs in a variety of animal species, including the mouse, rat, and rabbit. Because the latter species are small and can be maintained economically in the laboratory, their tumors have formed the bulk of material used in cancer research. Unfortunately, however, there are wide differences between animal cancer and human cancer, and there is no surety that findings obtained in mice can also be applied to man.
>
> "Obviously, the proper material for the study of human cancer is human cancer, but just as obviously, experimentation in man is intolerable. The next best bet would appear to be work with human cancer growing in laboratory animals.... Pertinent investigations were instituted in the Laboratory of Pathology at Yale and it was found that human cancer could be readily transplanted to lower animals....
>
> "The behavior of embryonic tissues transplanted to adult animals of the same as well as of alien species is under continued study.... It has been noted, for example, that transplanted embryonic tissues are much more susceptible to cancer-producing chemicals than are their adult counterparts -- responding both in a higher percentage of cases and in a shorter period of time.
>
> "Thus they form an excellent material for the testing of suspected carcinogens. It is of passing interest, in view of the present tobacco-lung cancer scare, that in innumerable experiments utilizing this technique, there has been no indication whatsoever of a causal relationship between tobacco and cancer."
>
> (The materials tested included extracts of pipe tobacco, cigarette tobacco, cigarette papers, and tar from pipes. More than 300 animals were used in testing the tobacco products, and the majority were held as long as a year.)

V-B 2

NO LUNG TUMORS PRODUCED IN HAMSTERS WITH TOBACCO TAR

Giuseppe DELLA PORTA, Leonard KOLB, M.D., and Philippe SHUBIK, D.Phil.,* tested the effect of known and suspected carcinogens on the respiratory mucosa of hamsters in 1958 (6-d) and reported:

> "The materials used in our studies were 9,10-dimethyl-1,2-benzanthracene and a cigarette tobacco tar condensate.... The materials were instilled directly into the tracheobronchial tree... via the oral route... once or twice a week, according to the experiment....
>
> "In experiments with repeated endotracheal instillations of 9,10-dimethyl-1,2-benzanthracene in a colloidal solution, hyperplasia, squamous metaplasia, atypical changes of the tracheo-bronchial epithelium, squamous-cell and adeno-carcinomas of the trachea and bronchi have been produced.
>
> "Administration of tobacco tar, also in colloidal suspension, produced no histologically demonstrable lesions."

*Of the Division of Oncology, Chicago Medical School, Chicago, Ill.

NO CIGARETTE "TAR" TUMORS IN HAMSTER POUCH

Condict MOORE, M.D., and A. James MILLER, M.D., of the Departments of Surgery and Pathology at the University of Louisville School of Medicine, Kentucky, in 1958 (37-m) reported details of their tests of cigarette smoke condensate wads implanted in hamster pouches. They say in part:

> "Of the 80 hamsters treated with tar wads, 55 survived treatment for a year or more. Of these, 40 were treated for over 18 months, and 10 were treated for 24 months or more. One animal survived treatment for 30 months. In these tar-treated animals no tumors developed. The histologic changes... consisted of thickening of the epithelium, slight chronic inflammatory reaction, thickening of the lamina propria, and occasional hyperplasia of the basal-cell area....
>
> "We know that human mouth cancer occurs occasionally in the complete absence of the use of tobacco. Therefore, tobacco is not an essential factor in producing squamous-cell cancer of the mouth. Furthermore, while epidermoid mouth cancer nearly always occurs in the presence of tobacco, millions of people do use tobacco in various forms and to various degrees and never acquire mouth cancer."

V-B 4

TOBACCO & SNUFF PRODUCED NO CANCER IN HAMSTERS

Erle E. PEACOCK Jr., M.D., and Bob W. BRAWLEY, B.S., of the Department of Surgery, University of North Carolina School of Medicine, and North Carolina Memorial Hospital, at Chapel Hill, in 1959 (10-p) reported the testing of implants of tobacco, snuff and other compounds in hamster pouches. They say:

> "Recognizing that animal experiments cannot answer the question entirely for human beings, but hoping to contribute additional experimental evidence, we have applied commercial snuff and tobacco directly to the oral mucosa of hamsters during a major portion of their life span....
>
> "The only animal in our series which had a known carcinogen (3-methylcholanthrene) implanted for 12 months, developed a highly anaplastic tumor... (which) has been transplanted into the submucosa of other hamsters for two generations. Oral mucosa of hamsters, therefore, issusceptible to local carcinogens and we have subsequently implanted a variety of other carcinogens to further evaluate the sensitivity and reactivity of this tissue....
>
> "Twenty-one snuff-implanted hamsters survived more than 13 months. Ten are still alive 18 months following implantation and 11 died between 13 and 18 months and were autopsied.... None of the hamsters developed a neoplasm of any type in either the snuff or control pouch.
>
> "Twenty-one tobacco-implanted hamsters survived more than 12 months and 16 of the animals are still living 18 months following implantation. Five died and were autopsied between 13 and 14 months after the implantation of tobacco. None of the tobacco-implanted or control pouches have developed a neoplasm, and none of the living animals show neoplastic changes."

ANTI-CANCER ACTION OF CIGARETTE SMOKE & TAR

Howard E. HOFFMAN, now at the Stine Laboratory, E. I. du Pont de Nemours and Co., Newark, Del., and A. Clark GRIFFIN, Department of Biochemistry, the University of Texas M.D. Anderson Hospital and Tumor Institute of Houston, in 1958 reported the action of cigarette tar and smoke in retarding cancer.* They say:

> "It was of interest to determine if exposure to cigarette smoke or smoke tar preparation would either enhance or inhibit the course of a well-established carcinogenic sequence, namely that resulting from the feeding to rats of diets containing the liver carcinogens 2-diacetylaminofluorene (DAF) or 3'-methyl-4-dimethylaminoazobenzene (3'MeDAB).
>
> "Within certain conditions, a definite delaying action of cigarette smoke and tar was found on the development of hepatic malignancy induced by 2-diacetylaminofluorene. In contrast, the precancerous state induced by 3'MeDAB was not inhibited by the use of tobacco tars or smoke....
>
> "The mechanism of the inhibition of tobacco tar can only remain a subject for speculation at this time. Miller, McDonald and Miller (Cancer Research 1:32, 1954) have found a similar inhibition of 2-acetylaminofluorene carcinogenesis by methylcholanthrene.... (63-m)
>
> "An alternative explanation of the inhibition is the presence of non-carcinogenic antagonists to DAF in tobacco tar. Numerous examples of non-carcinogenic inhibitors exist in the literature (reviewed by Crabtree, Brit. Med. Bull. 4:345, 1947)." (38-c)

* (27-h)

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C. Species Differences

ALL ANIMALS DO NOT REACT SIMILARLY TO "CARCINOGENS"

R. E. ECKARDT, M.D., Ph.D., FACP, Director of the Medical Research Division of the Esso Research and Engineering Company, of Linden, N.J., some years ago investigated the possible risks to workers exposed to isopropyl oil, and engaged laboratory experts to test its possible carcinogenicity in animals of various sorts. Dr. Eckardt is also Associate Clinical Professor of Industrial Medicine at New York University Postgraduate Medical School and Instructor in Medicine at Cornell University Medical School. In a 1959 paper (4-e) he said:

> "It should be remembered that not all species will respond in the same way to the same material. Thus in some experiments performed for us several years ago, mice, rats, rabbits, guinea pigs and monkeys were all painted with the same material three times a week. Rats and guinea pigs developed nothing at all when painted throughout their life span. After several months, rabbits began to develop small tumors at the site of painting. With continued painting, these tumors increased in size and number, but very few of them became malignant. Mice developed small papillomas in about 40 days and with continued painting, many of these became malignant before 150 days.
>
> "Monkeys developed small papillomas after about a year of painting. After eight years of painting, one of these animals had a tumor that had all the gross characteristics of a malignancy. Microscopically it also looked like a malignancy, yet it did not metastasize, grew in size very slowly and had few of the biological characteristics of malignancy, even though morphologically it looked like cancer.

"The results of these tests quite properly raise the question of which result if any is appropriate for the evaluation of the safety of food additives. Yet today there are those who state unequivocally that skin painting in mice is an appropriate test for materials which are ingested or inhaled, ignoring the results in other species. Quite frankly, I must say, I don't know the answer to this important question. I am prepared to accept skin painting tests in mice as a guide to the probable activity of the same material on the skin of the human, but to extend this unhesitatingly to the lungs or gastrointestinal tract as this time seems quite inappropriate....

"Further, it has been observed that sugar and cholesterol when injected subcutaneously into rats and/or mice will produce sarcomas. Does this mean that these materials are carcinogenic in appropriate tests and should be excluded from the food supply? On the contrary, I believe that these results simply suggest that the subcutaneous tissues are highly inappropriate as test sites for evaluating the safety of food additives. Thus when someone says a material is 'carcinogenic,' it is essential to inquire in what species and by what type of test if any practical implications are to be drawn from the observations. It is not enough to say, as the American Cancer Society has said, that they are opposed to the incorporation of carcinogens in the food supply. This is like being opposed to sin. But like sin, a carcinogen needs a definition. One academic definition that has been proposed is that a carcinogen is any material which produces a tumor at any dosage in any species by any route of administration. With such a definition of a carcinogen, Public Law 85-929 (Food and Drug Law Amendments) becomes impossible to administer....

"There are no magic formulas, no crystal balls, or simple slide rules that will permit the public health official to come up with the answer to the question of whether a given compound will present a cancer hazard to the general public if it is permitted to enter the food supply. Life would be very simple for all of us if there were such a magic formula. The decisions on such questions will have to be reached after weighing all the available evidence for and against the compound under consideration.... Remember that 3,4-benzpyrene is probably present in our drinking water at concentrations of one part per billion....

"Because there is so much emotion associated with the word 'cancer' today, people are likely to be panicked into hasty emotional decisions where carcinogens are concerned.... Much more basic, fundamental research needs to be undertaken...."

Richard H. BARNES, Ph.D., in a 1959 paper (7-b) prepared with the cooperation of the Faculty of the Graduate School of Nutrition at Cornell University, describes the numerous species differences encountered in animal research.

> "If we seek an understanding of a disease process or a treatment of that disease, the final answer can only come from the clinical patient. This must not be interpreted as a justification to abolish or reduce animal experimentation. Rather, these examples of lack of correlation should emphasize the need for the investigator to establish firmly the fundamental cellular or subcellular biochemical metabolic phenomena that are involved. Then, as he utilizes the intact animal to seek out non-metabolic possibilities that might cause species differences, he can extend his studies to a wider assortment of animals in the hope of finding parallelisms that will be helpful. However, he should always tread cautiously in interpreting the results he obtains in the laboratory."

Dr. Philippe SHUBIK and his associates at Chicago Medical School in 1957 reported on cancer development in untreated mice (20-s). Eight of 25 untreated albino mice developed multiple papillomas, sebaceous adenomas, and carcinomas, although exluded from contact with any possible carcinogenic contamination.

V-C 5

INCIDENCE AND ETIOLOGY OF LUNG CANCER

Seymour M. FARBER, M.D., associate clinical professor of medicine at the University of California Medical School, San Francisco, wrote a book on "Lung cancer" in 1954 (3-f). He discusses etiology in part as follows:

> "The record of the medical profession in the diagnosis of bronchogenic carcinoma is regarded as unsatisfactory. Our own findings are in comformity with published studies. We have surveyed 1070 cases of bronchogenic carcinoma, morphologically proven, collected from 19 hospitals in California. In 61% of these cases, no positive diagnosis was made prior to autopsy. Compared year by year, it apparently took as long to make a correct diagnosis of the disease in 1949 as it had in 1935....
>
> "Cancer producing substances are not only specific for certain laboratory animals, and strains within the genus, but are very often active only in a particular organ of the affected animals. Cancer arises as a result of an interaction between the specific tissue of the host and the carcinogenic agent.
>
> "The pronounced age and sex predilections of bronchogenic carcinoma suggest just as clearly the importance of hormonal factors....
>
> "Although the relationship between exogenous agents, and the native constitution of the individual to date has proven to be almost impenetrable, it is obvious that constitutional factors are important.... The present tendency is to see the important factor of 'native constitution' as reflecting the complicated system of hormones and related substances which govern physiology....
>
> "Where the disease occurs in children, it seems that the dominant consideration is the hormonal one.... Perhaps in all cases both factors are in play, but they do not have a consistent relationship to one another."

V-D

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D. Applicability to Humans

Willard MACHLE, M.D., Associate Clinical Professor of Industrial Medicine, New York University-Post Graduate Medical School, said in a paper published in 1956 (8-m):

> "It has been stated that approximately 25% of all new chemical compounds tested on the mouse or in the rat can be shown to be carcinogenic.... The subcutaneous injection of ground glass, certain plastics and even glucose, into the rat, will induce sarcomata. Are we to conclude from this that these materials are likewise carcinogenic for man?
>
> "Would we be better justified, perhaps, in reasoning by analogy from the production of epidermoid carcinomata of the lung of rats to a corresponding effect in man? To my knowledge, there is no evidence that the epidermoid carcinoma of the lung in the rat is initiated by the same route or develops like the epidermoid carcinoma of the lung of man....
>
> "We ought to keep in mind the limitations of the experimental method as applied to carcinogenesis and be properly cautious in the translation of effects produced in animals to assumed effects on man."

Seymour J. KRESHOVER, M.D., then at the Medical College of Virginia School of Dentistry, reported on the effects of malnutrition on experimental carcinogenesis in animals in 1955 (10-k). He said:

> "Vitamin B complex deficiency in mice renders their cutaneous tissues more susceptible to the irritating effects of whole tobacco smoke....
>
> "Vitamin B complex-deficient hamsters, currently under study, show no significant cutaneous tissue changes even after receiving twice as many smoke applications as did the mice. This indicates the questionable validity of making conclusions on the basis of experiments with a single series of laboratory animal, and the speculation involved in applying such findings to man."

CARCINOGENIC EFFECTS OF THERAPEUTIC AGENTS

T. H. C. BARCLAY, M.D., of Regina, Saskatchewan, Canada, in 1960 (58-b) reviewed the data on substances which are generally considered to be innocuous or safe to administer in therapeutic doses which have been found to have a carcinogenic effect in some species of experimental animals. He says:

> "When the subject of malignant disease is being dealt with the emotional factors involved often blunt critical faculties. The need for perspective in the evaluation of experimental data in animals and their relationship to cancer in humans is long overdue. Many drugs which have found widespread acceptance in clinical medicine and are extremely valuable therapeutic agents have been shown to produce a carcinogenic effect under certain laboratory conditions.....
>
> "Moreover, of the so-called 'innocuous' substances which have been shown to be carcinogenic in rats and mice, sodium chloride, glucose, fructose, galactose, and lactic acid are included.
>
> "In contrast to these findings with therapeutic agents, Szepsenwol (56-s) reported a high yield of tumors in 16 mice who were maintained from the age of three months on a diet supplemented with hard-boiled eggs.... It is not suggested that as a result of this work eggs should not be ingested by man."

The NEW SCIENTIST, of London, England, said editorially in 1959 (5-m):

> "The frequently found failure to produce cancer in experimental animals by cigarette smoke also suggests that the link between lung cancer and smoking may not be simple due to the presence of a cancer-producing substance in the smoke, but that other factors may play an important part.
>
> "Only in the Sloan-Kettering Institute in New York has a high incidence of cancers been produced by the tarry products in cigarette smoke. These experiments have now been published in detail, and it has become obvious why they succeeded while others failed. The amount of tar needed is quite phenomenal, and bears no relation to the human case. With the amounts used by the other workers no cancers were produced."

Philippe SHUBIK, Dr. Phil., and Jean SICE, of the Chicago Medical School, reported on chemical carcinogens as a chronic toxicity test in (19-s). They said:

> "It is sometimes not possible to relate an isolated chemical substance to the biological activity of a mixture, as illustrated by the carcinogenic action of coal tar. 3,4-benzpyrene has been isolated from coal tar and shown to be a potent carcinogen for the skin of the mouse, although it is incomparably less active on the skin of the rabbit than tar itself. Actually, a fraction of coal tar, free of benzpyrene, is carcinogenic to the skin of the rabbit, although not to that of the mouse.
>
> "The compound responsible for tar cancer in man has not been identified, and there is no proof, as is sometimes assumed, that 3,4-benzpyrene is carcinogenic to man.... There is no way yet of deciding the correct species to be used for a particular carcinogenicity test, since results in the mouse, rat, or any laboratory animal may possibly have no bearing on the human hazard."

V-E

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E. Chemistry of Smoke

CHEMICAL COMPONENTS OF TOBACCO AND SMOKE

A review of current knowledge on the chemical constituents of tobacco and tobacco smoke by Robert A. W. JOHNSTONE and Jack R. PLIMMER of the British Medical Research Council, stationed at the University of Exeter, England, (in 1959) summarized the data from 419 scientific papers in many languages (4-j). In commenting on the aromatic hydrocarbons they said:

> "The emphasis on the polycyclic aromatics in smoke has arisen because of its supposed connection with lung cancer and the known carcinogenic activity of some of the higher polycyclic hydrocarbons. Nevertheless, because of the low concentration of these compounds in tobacco smoke it has not been possible to isolate and identify them in the usual way....
>
> "It would be desirable to have further proof of the existence in tobacco smoke of many of these compounds. The highly potent carcinogen, 3,4-benzpyrene, has received a large proportion of the attention paid to polycyclic hydrocarbons in smoke and proof of its presence, or that of a derivative, seems fairly conclusive with regard to chromatographic and spectroscopic evidence (59-b), although the amount is extremely small."

V-E 2

HYDROCARBONS IN TOBACCO TOO MINUTE TO ACCOUNT FOR CARCINOGENESIS

Jytte AHLMANN, of the Pathological-Anatomical Institute of the University of Copenhagen, Denmark, reported on delicate microanalytical procedures applied in determining the chemistry of cigarette smoke in 1958 (23-a):

> "By means of the methods used the presence of 38 different substances was ascertained in the neutral and acid fraction of cigarette tar. For some of these substances (altogether ten) further characterization has not been possible, chiefly because the quantities available were too small.
>
> "Among the hydrocarbons present special mention should be made of 3,4-benzpyrene, 1,2-benzanthracene and 6,7-cyclopenteno-1,2-benzanthracene, since these substances are known to be carcinogenic. About two micrograms 3,4-benzpyrene was found in the tar from 1000 cigarettes.*
>
> "It has thus been shown that cigarette tar contains a great number of polycyclic hydrocarbons, but in such low concentrations that none of them are able, presumably, to account for any direct carcinogenic effect.
>
> "Whether the aggregate action of these substances may contribute to a co-carcinogenic action may be ascertained through biological experiments."

*(Ed. note: two parts per billion. As an indication of how much 1,000,000,000 really is, slightly over one billion minutes have elapsed since the start of the Christian era.)

V-E 3

DISCREPANCY IN AMOUNTS OF BENZPYRENE IN AIR AND SMOKE

Leiv KREYBERG, of the Institute for General and Experimental Pathology of the University of Oslo, Norway, in 1959 (23-k) undertook to determine the amount of 3,4-benzpyrene (carcinogenic to mouse skin in high concentrations) at two gasworks at sites where workers are regularly employed. He said:

> "The first comment to these findings is to stress that they do not all intend to give a complete picture of the air pollution in such plants. The report actually represents a pilot study and should be followed up with more extensive and more complete investigations.
>
> "With the coarse technique used, the figures indicate that the air in the retort house of Oslo Gasworks contains 3,4-benzpyrene in amounts corresponding to some 5000 cigarettes daily for a worker with a 40 hour week. Even if the figures for Bergen are lower (200/730), they are nevertheless of the same order of magnitude and the 'cigarette-equivalent' is enormous.....
>
> "If these two facts are correlated: the enormous amounts of 3,4-benzpyrene in the air and the very moderate excess of lung cancer in the gasworks, a serious fallacy is evidently involved....
>
> "The main support for the assumption that 3,4-benzpyrene is an important factor in the increased development of lung cancer is its immediate plausibility. The substance is present in polluted town air and in tobacco smoke and the substance is carcinogenic in animals. But 3,4-benzpyrene is present in cigar and pipe smoke in even greater concentrations than in cigarette smoke... but not connected with anything like the same risk for lung cancer development. The role of 3,4-benzpyrene in the development of lung cancer is very far from known."

V-E 4

MINUTE AMOUNTS OF HYDROCARBONS FOUND IN SMOKE

Benjamin L. Van DUUREN, Sc.D., and associates at the Institute of Industrial Medicine, New York University-Bellevue Medical Center, in 1960 (8-v) described techniques used in the reported identification in cigarette smoke condensate of ten compounds listed as carcinogens.

In the table below are the chemicals concerned, the concentration reported by the authors, and the Editor's calculation of this concentration in terms of parts per billion of the tobacco.

Compound	Micrograms per 100 c'ettes	Parts per Billion
3,4-benzpyrene	0.50	5
1,2-benzpyrene	0.30	3
1,2;7,8-dibenzacridine	0.27	2.7
3,4;5,6-dibenzcarbazole	0.07	.7
Chrysene	0.06	.6
1,2;5,6-dibenzanthracene	0.05	.5
1,2;5,6-dibenzacridine	0.01	.1
3,4;9,10-dibenzpyrene	0.002	.02
3,4-benzphenanthrene	---	Traces
7,8- or 10,11-benzfluoranthene	---	Traces

The authors say:

> "Skin-painting experiments carried out in this laboratory (unpublished results with mice) on the basic fraction of cigarette tar were negative; however, it is now clear on the basis of the data reported here that the concentration of the basic fraction used in these experiments would not have been sufficient to yield skin tumors even if the dibenzacridines had been as potent for the skin as 3,4-benzpyrene, which is not the case."

BIOCHEMICAL RESULTS NOT PRECISELY MEANINGFUL

George F. WRIGHT, Ph.D., of the University of Toronto, Canada, discussed the significance of mouse-skin tests for carcinogenicity of tobacco smoke condensate in 1960 (39-w) as follows:

"Once one sets up a large scale chromatographic separation, as was done at Toronto in order to separate the smoke condensate (i.e. tar) from 3000 lb. of flue-cured tobacco a large number of polynuclear aromatic compounds may be drawn off, rechromatographed and otherwise treated for purification, and identified. Among these hundreds of aromatic compounds some will be carcinogenic toward mouse skin.

"At first the effort was directed to find enough carcinogens to account for the mouse-skin activity in the entire smoke condensate. We failed to accomplish this objective despite great care to prevent the destruction by light and air of the carcinogens. It should be understood at this point that hitherto-unknown carcinogens of oxygen sensitivity greater than is commonly encountered might have been lost because of the limitations of known techniques as of 1957-1958. At any rate we were unable to find sufficient carcinogenic activity to account for that of the entire smoke condensate.

"During this same period we examined each fraction from the chromatographic separation for the presence of a 'supercarcinogen' which might account for the activity of the complete tar but without success.

"Therefore we are faced with the situation where we cannot match the whole with the sum of its parts. Several explanations are possible:

"Carcinogenic polynuclear aromatic hydrocarbons

"1. have been destroyed during the process, or

"2. a totally unrelated carcinogen has been destroyed.

"3. Symbiosis is operative and the ratio of the symbiotic members has been altered.

"4. Stating (3) in another way a co-carcinogen has been lost.

"5. The effectiveness of polynuclear aromatic carcinogen does not follow an additive relationship.

"The first four of these possibilities are subjects of study by chemists or by chemists and physiologists together. However the fifth, which is the most significant has never to my knowledge been answered by physiologists. Until it is answered any answer to the tobacco carcinogen problem which is based on physiological test must necessarily be vague and questionable. This is so because the 'polynuclear aromatic spectrum' of compounds changes appreciably with differences in tobacco combustion, with differences in tobacco and with differences in cure. If the effectiveness of polynuclear aromatic carcinogens is not additive then the composition, i.e. the 'spectrum of compounds' assumes an importance which may be as great, or even greater than the cumulated percentage of carcinogens.

"Let me state the problem of (5) in another way. If every carcinogen affected the nucleus of a cell in the same manner during the lifetime of that cell and in a variety of environments then we can reduce the measure of effectiveness simply to the action of an 'average carcinogen' on 'an average cell' in an 'average time' and the measure is simply one of additivity. But if carcinogen I affects cell A at time M while carcinogen II affects cell A at time N then 2 microgram of either I or II will be less effective than 2 micrograms comprised of 1 microgram of each of carcinogens I and II. That is to say that cell A can be twice vulnerable when two carcinogens are present but only once vulnerable when only one (time-limited) carcinogen is present. Alternatively if the melange of cells contains cells A and B but A is more susceptible than B to carcinogen I (and vice-versa with respect to carcinogen II) then a mixture of I and II is likely to be more effective than either I or II in quantity equal to that of the mixture. In other words the effect of carcinogens will not be additive.

"There are several other variations of the same theme, but they all point to the fact that the physiologist's test method for mouse-skin carcinogenicity is not sufficiently developed for quantitative use by the chemist. The physiologist cannot be condemned for this inadequacy, because the problem is great; indeed if he knew all of these answers he would know a great deal more about carcinogenesis than now is known. Nevertheless the chemist cannot give a meaningful answer to the significance of the carcinogens which he isolates except in the crudest sense. As professional chemist I refuse to report my results publicly as precisely meaningful under these circumstances."

Other comments on smoke chemistry include:

"It is difficult to assess the usefulness of such experiments (applying smoke condensates to mice backs) in the interpretation of the relationship between cigarette smoking and cancer of the lung in man. It is known that cigarette smoke contains very slight traces of substances such as benzpyrene which are carcinogenic to mouse skin, and it is not therefore surprising that when smoke is concentrated to a viscous fluid it will show some degree of carcinogenic activity when applied to mice."

T. D. DAY, M.D., 1959 (23-d)

"Among 72 mice in the unfiltered atmosphere 11 developed one or more lung adenomata. Among 74 mice in the soot-free atmosphere, 16 developed one or more lung adenomata. The mice in the soot-free atmosphere lived on average about 100 days longer than the other group and so were at risk for this additional time.... Genetic constitution undoubtedly plays a great part in determining the incidence of tumors in line-bred mice.... What determines the site at which tumors arise is still unexplained."

P. R. PEACOCK, M.B., FRFPSG, 1959 (38-p)

For further discussion of the results of smoke inhalation tests in animals see 16-a, 74-b, 35-b, 13-e, 45-h, 46-h, 33-h, 20-k, 9-l, 10-l, 20-l, 54-m, 55-m, 73-p, 39-p, 40-p, 57-s

For other papers on cancers produced on mouse skins with common alimentary, medicinal and other substances considered innocuous to man see 17-a, 18-a, 9-a, 61-b, 62-b, 63-b, 24-d, 29-g, 47-h, 48-h, 49-h, 21-k, 8-m, 56-m, 57-m, 9-n, 10-n, 9-o, 10-o, 41-p, 31-r, 32-r, 56-s, 58-s, 59-s, 12-t

For further discussion on species susceptibilities and spontaneous carcinogenesis see 19-a, 4-a, 11-a, 20-a, 21-a, 64-b, 20-b, 42-b, 65-b, 13-e, 30-g, 52-h, 38-h, 50-h, 28-h, 33-h, 35-h, 8-k, 1-l, 16-l, 17-l, 19-l, 58-m, 59-m, 17-p, 42-p, 43-p, 60-s, 19-s, 20-s, 61-s, 62-s, 44-s, 11-w

For other papers commenting on the question of the applicability of animal tests to human disease see 22-a, 13-c, 13-e, 3-e, 18-f, 17-g, 38-h, 51-h, 33-h, 34-h, 35-h, 10-k, 13-k, 1-l, 34-l, 23-l, 5-m, 29-m, 30-m, 5-n, 5-o, 44-p, 34-r, 70-s, 17-s, 18-s, 19-s, 63-s, 54-s, 11-w

For further discussion of the quantities and characteristics of substances identified in cigarette smoke condensate see 37-c, 14-f, 23-k, 18-s, 19-s, 40-w

For papers on negative results of tests with tobacco products on various animal species and transplants see 74-b, 76-b, 41-c, 33-f, 34-f, 28-g, 16-g, 17-g, 67-m, 41-r, 74-s, 14-t, 43-w

For papers on the inhibitory effects of tobacco products on tumors, bacteria, and infections see 25-a, 75-b, 77-b, 10-j, 68-m, 55-p, 15-t, 10-v, 44-w